

Application No.: 09/647,661

Attorney Docket No.: DALHO1270-2

Filing Date: January 31, 2001

(028614-1001)

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Amendments to the Claims

Please amend claims 1, 6, 18, 24 and 31 as indicated in the listing of claims below.

Please add new claims 38-44 as presented in the listing of claims below. Please cancel claims 7-12, 26 and 27 without prejudice.

Listing of claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A conjugate comprising a targeting compound and a nitroreductase, said nitroreductase having:
 - (a) a pI greater than about 6.0,
 - (b) 2 or more cysteine residues, and
 - (c) a preference for NADPH as an electron donor;wherein said nitroreductase is capable of converting metronidazole ~~a prodrug~~ to one or more cytotoxic compounds.
2. (Original) A conjugate according to claim 1, wherein said targeting compound is covalently linked to said nitroreductase.
3. (Original) A conjugate according to claim 1, wherein said targeting compound is an antibody.
4. (Original) A conjugate according to claim 3, wherein said antibody is a monoclonal antibody.

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5. (Original) A conjugate according to claim 3, wherein said antibody is specific for tumor cell surface antigens, precancerous cell surface antigens, cell surface antigens characteristic of autoimmune diseases, selected tissue-specific antigens or selected organ-specific antigens.

6. (Currently amended) A conjugate according to claim 1, wherein said metronidazole is prodrug is a compound used to treat *Helicobacter* infections.

7. - 12. (Cancelled).

13. (Original) A conjugate according to claim 1, wherein said nitroreductase is isolated from a microaerophilic bacterium, said microaerophilic strain having a sensitivity to nitro-containing compounds with a redox potential in the range of about -500mV to about -350mV.

14. (Original) A conjugate according to claim 13, wherein said microaerophilic bacterium is *Helicobacter*.

15. (Original) A conjugate according to claim 13, wherein said microaerophilic bacterium is *Camphylobacter*.

16. (Original) A conjugate according to claim 13, wherein said microaerophilic bacterium is an *H. pylori* strain.

17. (Original) A conjugate according to claim 16, wherein said *H. pylori* strain is HP950.

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18. (Currently amended) A nitroreductase having:

- (a) a pI greater than about 6.0
- (b) 2 or more cysteine residues,
- (b) a preference for NADPH as an electron donor; and

wherein said nitroreductase is capable of converting metronidazole a prodrug to one or more cytotoxic compounds.

19. (Original) A nucleic acid encoding the nitroreductase of claim 18.

20. (Original) A nucleic acid having greater than about 90% homology to the ORF in SEQ ID NO: 1.

21. (Original) A nucleic acid according to claim 19, wherein said nucleic acid is expressed in a heterotypic cell.

22. (Original) A nucleic acid according to claim 21, wherein said heterotypic cell is a bacterium, a virus, a retro-virus, a yeast, or a eukaryotic cell.

23. (Original) A nucleic acid according to claim 22, wherein said bacterium is E. coli.

24. (Currently amended) A method for selectively killing or inhibiting the growth of target cells, said method comprising the administering of a conjugate according to claim 1, wherein administration of said conjugate is in conjunction with administration of metronidazole a prodrug, said prodrug having a redox potential in the range of about 500mV to about 350mV, and

wherein said nitroreductase converts said metronidazole prodrug into one or more toxic compounds.

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25. (Original) A method according to claim 24, wherein said target cells are selected from bacterial cells, viral cells, fungal cells, yeast cells, T-cells, B-cells, tissue cells, organ cells, diseased cells, tumor cells or neoplastic cells.

26. (Cancelled).

27. (Cancelled).

28. (Original) A pharmaceutical formulation comprising a nitroreductase according to claim 18, optionally conjugated with a targeting compound, and a suitable carrier.

29. (Original) A pharmaceutical formulation comprising a conjugate according to claim 1, and a suitable carrier.

30. (Original) A therapeutic method for delivering to a patient a pharmaceutical formulation according to claim 28.

31. (Currently amended) A method according to claim ~~24~~ 27, wherein said carrier is selected from liposomes, latex beads or microspheres. ~~32 A method for detecting plasmid loss by a bacteria, said method comprising~~

~~transforming said bacteria with a plasmid encoding a nitroreductase,~~

~~and~~

~~assaying for growth of said bacteria on a nitroaromatic-containing media;~~

~~wherein said nitroreductase, as inserted into said plasmid, is expressed in said bacteria, said nitroreductase having:~~

~~a pI greater than about 6.0~~

~~greater than 2 cysteine residues, and~~

~~a preference for NADPH as an electron donor;~~

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~~wherein said nitroreductase is capable of reducing said nitroaromatic compound to one or more cytotoxic compounds, and~~

~~identifying as having lost said plasmid, any of said transformed bacteria which grow on said nitroaromatic-containing media.~~

32. (Original) A method for detecting plasmid loss by a bacteria, said method comprising

transforming said bacteria with a plasmid encoding a nitroreductase,

and

assaying for growth of said bacteria on a nitroaromatic-containing media;

wherein said nitroreductase, as inserted into said plasmid, is expressed in said bacteria, said nitroreductase having:

a pI greater than about 6.0

greater than 2 cysteine residues, and

a preference for NADPH as an electron donor;

wherein said nitroreductase is capable of reducing said nitroaromatic compound to one or more cytotoxic compounds, and

identifying as having lost said plasmid, any of said transformed bacteria which grow on said nitroaromatic-containing media.

33. (Original) A method for identifying substrates for a nitroreductase according to claim 18, said method comprising

transforming a host cell with a plasmid encoding said nitroreductase,

and

assaying for growth of said host cell on a medium containing the putative substrate,

wherein said nitroreductase converts any substrate present in said medium to one or more cytotoxic compounds such that said transformed cells will be killed or growth-inhibited, and

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identifying as a substrate any of said putative substrates causing killing or growth-inhibition of said transformed cells.

34. (Original) A kit for identifying a bacterium that expresses a nitroreductase, said kit comprising a substrate for said nitroreductase, wherein said nitroreductase converts said substrate into one or more detectable products.

35. (Original) A kit according to claim 34, wherein said nitroreductase is the H. pylori rdxA gene product.

36. (Original) A kit according to claim 35, wherein said nitroreductase converts said substrate into one or more cytotoxic compounds.

37. (Original) A kit according to claim 36, wherein said substrate is metronidazole.

38. (New) A nitroreductase according to claim 18, wherein said nitroreductase is expressed in a heterotypic cell.

39. (New) A nitroreductase according to claim 38, wherein said nitroreductase is expressed using an expression vector comprising a nucleic acid having greater than about 90% homology to the ORF in SEQ ID NO: 1.

40. (New) A nitroreductase according to claim 38, further wherein said heterotypic cell is a bacterium, a virus, a retro-virus, a yeast, or a eukaryotic cell.

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41. (New) A method for selectively killing or inhibiting the growth of target cells, said method comprising administering a nitroreductase according to claim 18,

wherein administration of said nitroreductase is in conjunction with administration of metronidazole, and

wherein said nitroreductase converts said metronidazole into one or more toxic compounds.

42. (New) A method according to claim 41, wherein said target cells are selected from bacterial cells, viral cells, fungal cells, yeast cells, T-cells, B-cells, tissue cells, organ cells, diseased cells, tumor cells or neoplastic cells.

43. (New) A method for selectively killing or inhibiting the growth of target cells, said method comprising administering a nitroreductase according to claim 38,

wherein administration of said nitroreductase is in conjunction with administration of metronidazole, and

wherein said nitroreductase converts said metronidazole into one or more toxic compounds.

44. (New) A method according to claim 43, wherein said target cells are selected from bacterial cells, viral cells, fungal cells, yeast cells, T-cells, B-cells, tissue cells, organ cells, diseased cells, tumor cells or neoplastic cells.